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Figure 1. The Cycle of Fatty Acid Elongation in Bacteria

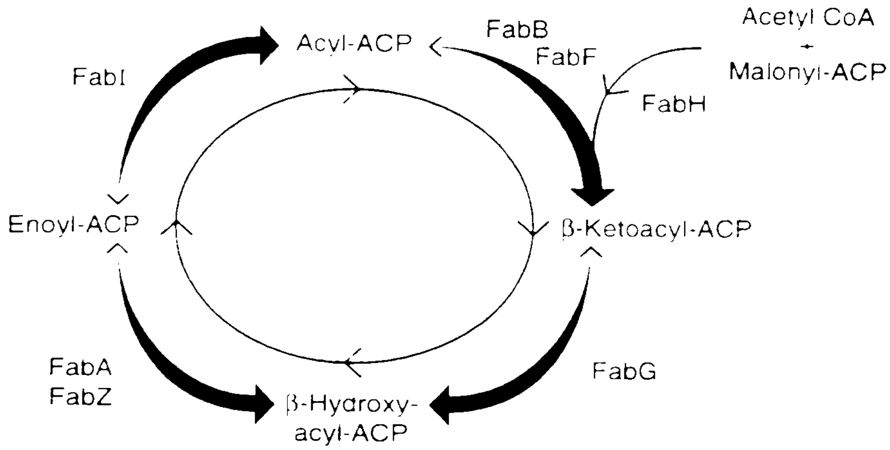

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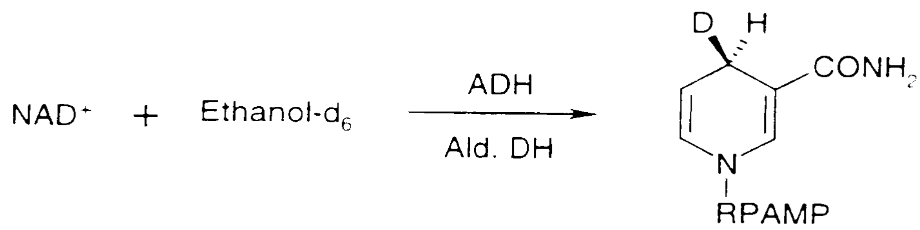
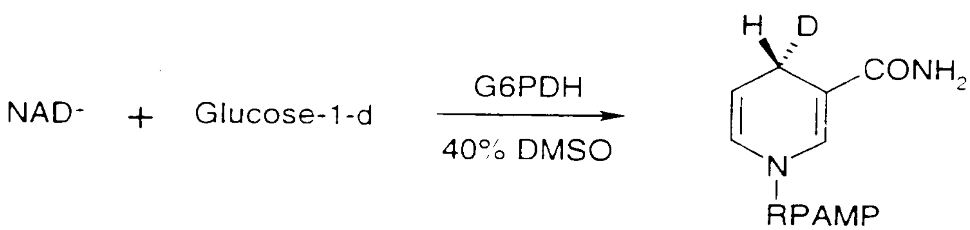
Figure 2. Synthesis of Deuterated Pyridine Nucleotides

Synthesis of R-NADD



[4R-^{2}H]NADH

Synthesis of S-NADD



[4S-^{2}H]NADH

Figure 3. Predicted (Observed) Product Structures and $(M+H)^{+}$'s

(Based on *E. coli* Fab I)

Reaction

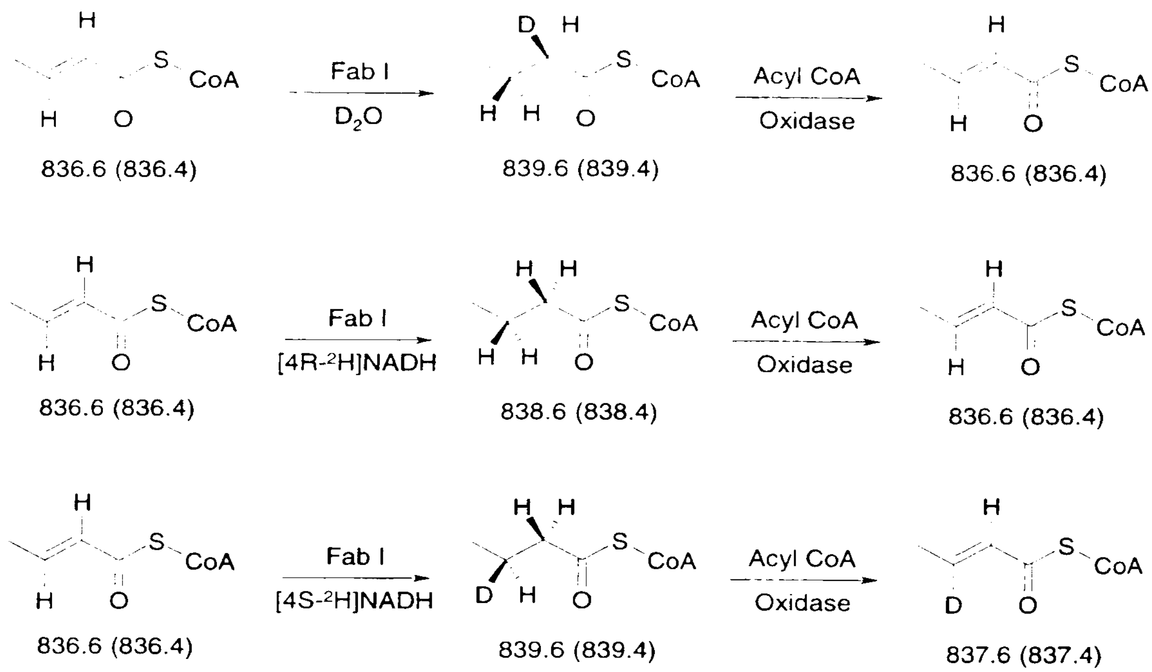


Figure 4. Mass Spectra of Components from Reaction 3

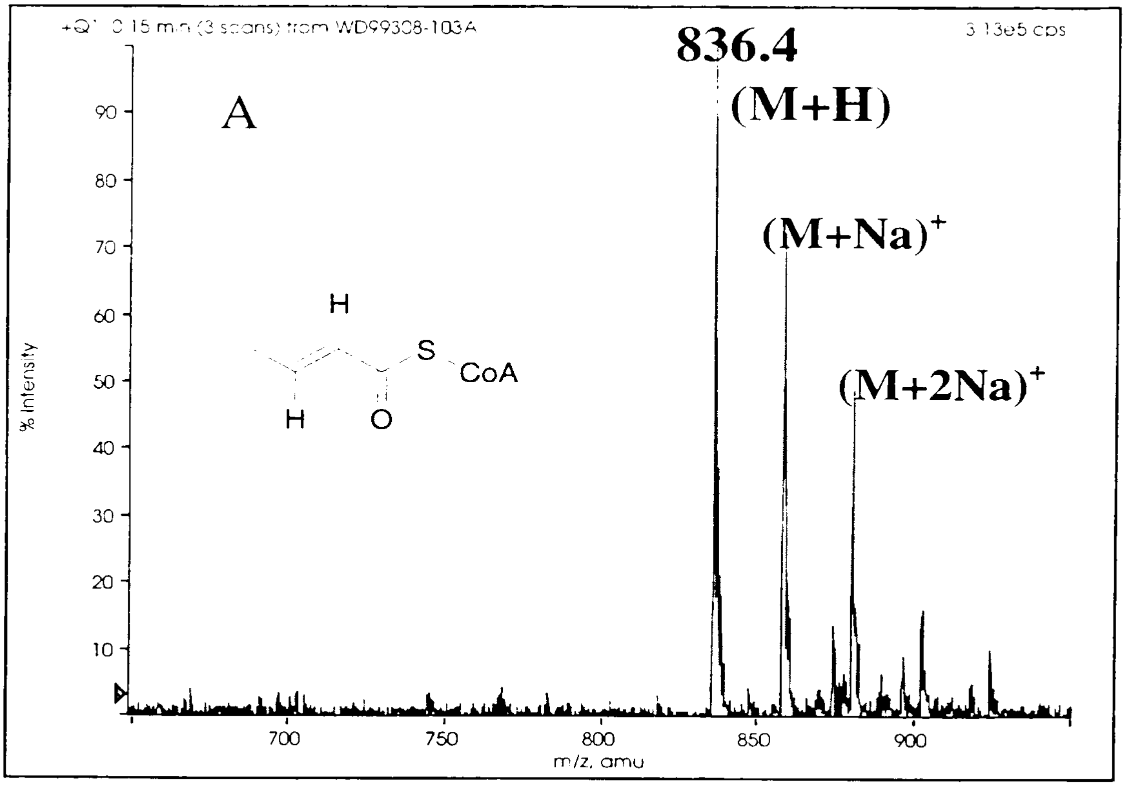


Figure 4 B

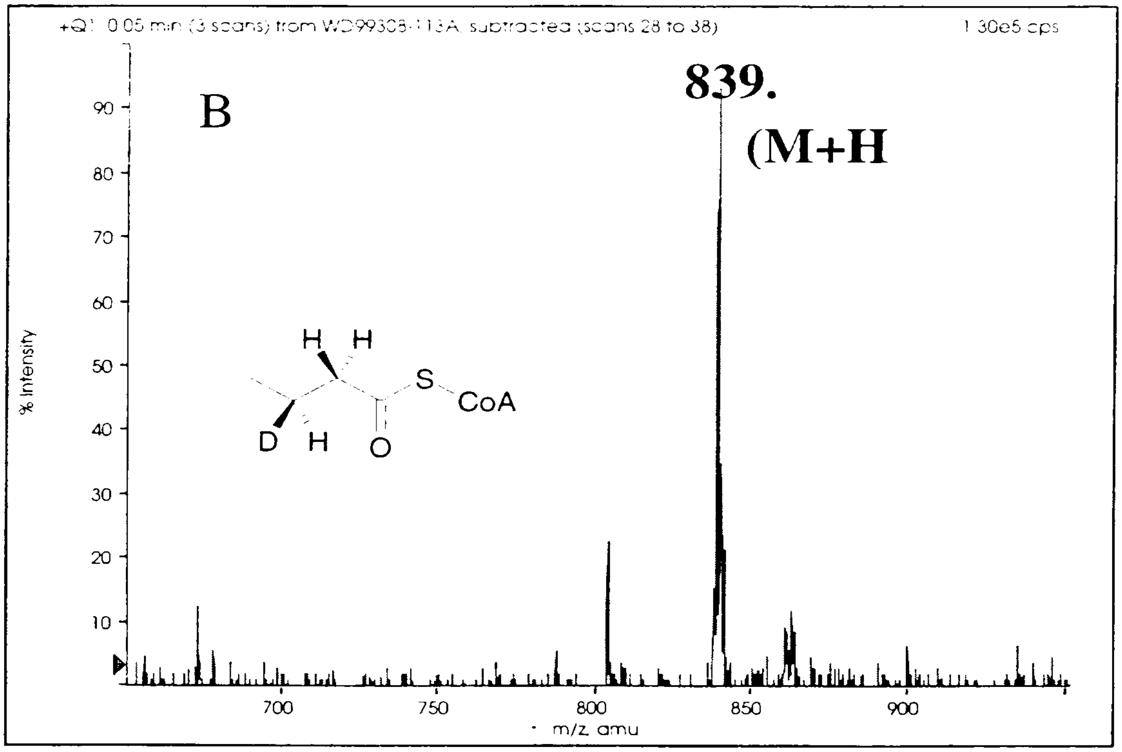


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Figure 4 C

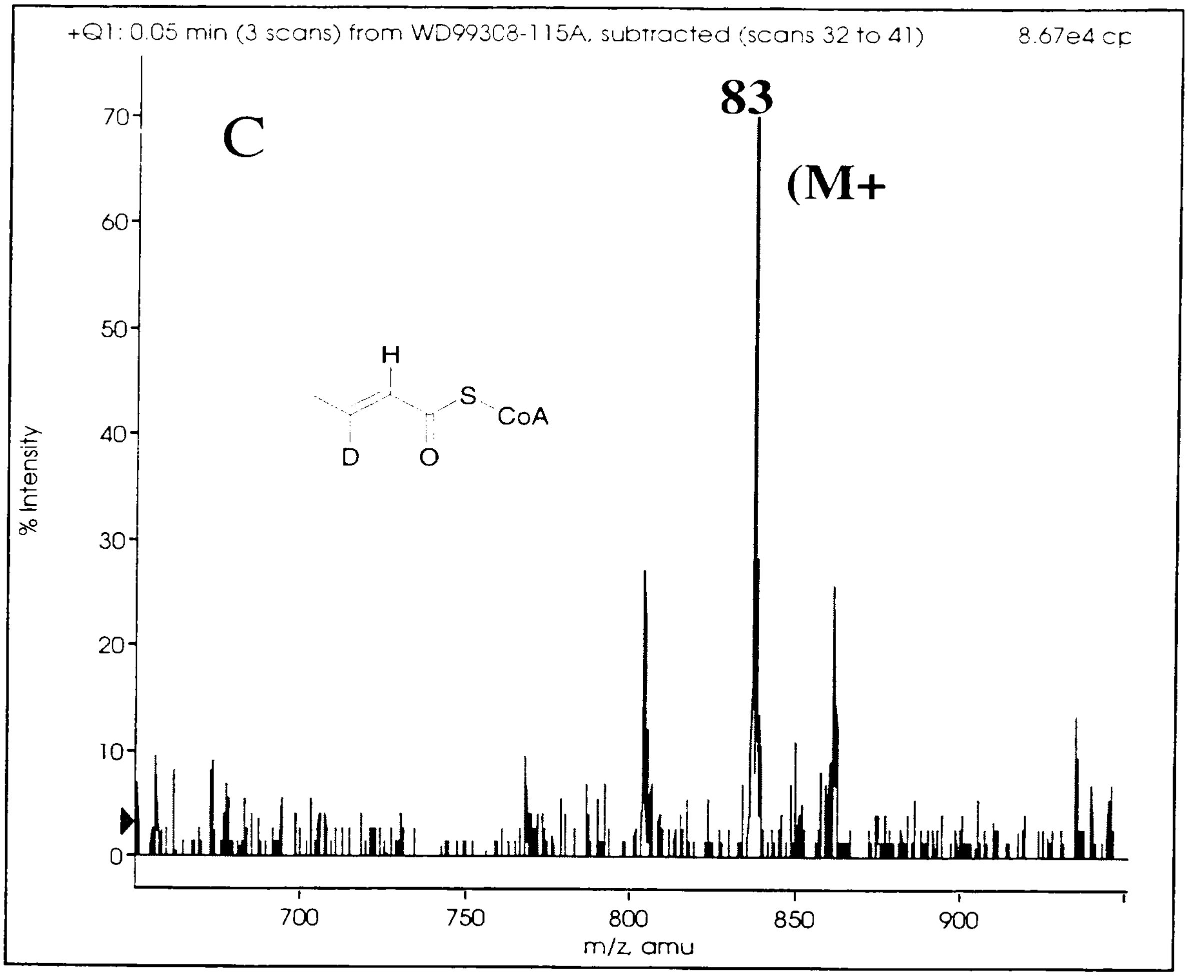

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Figure 5. Stereochemical Course of *S. aureus* Fab I

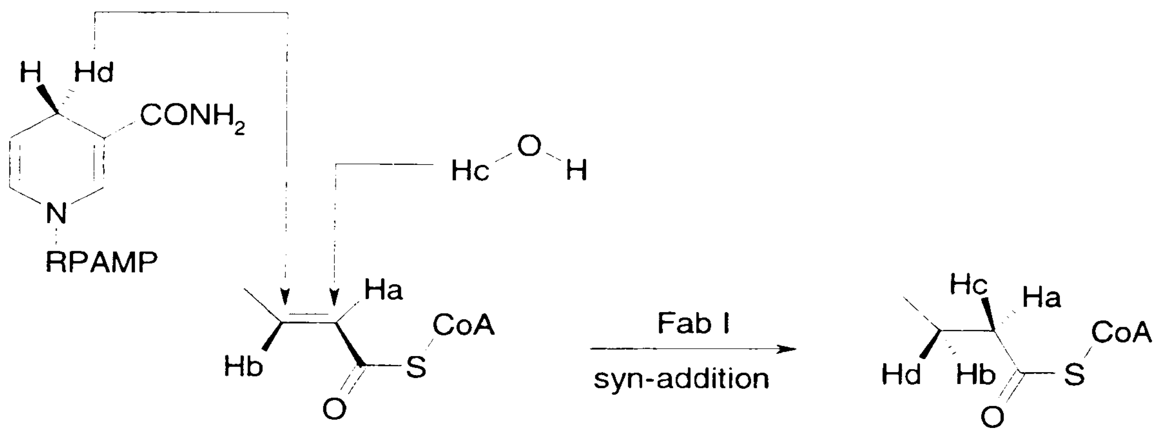
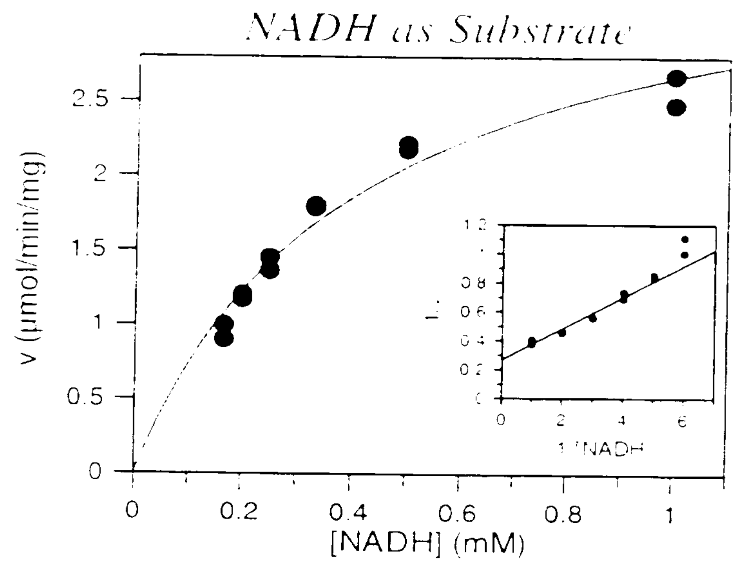
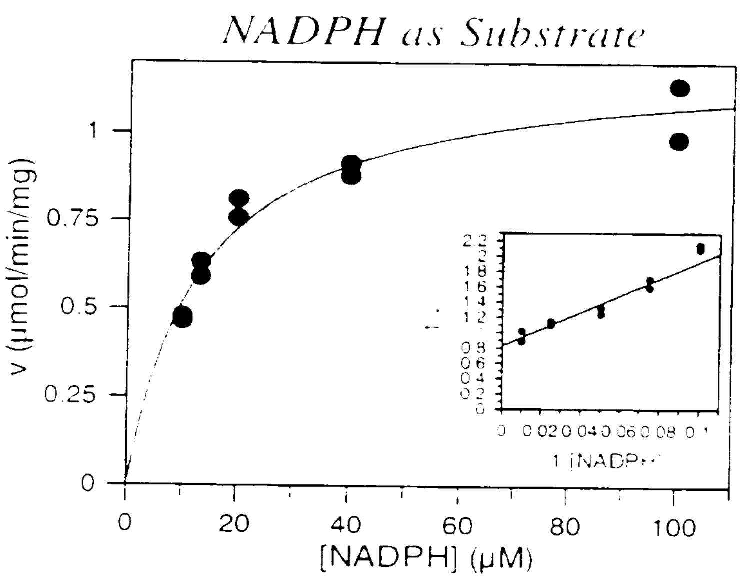


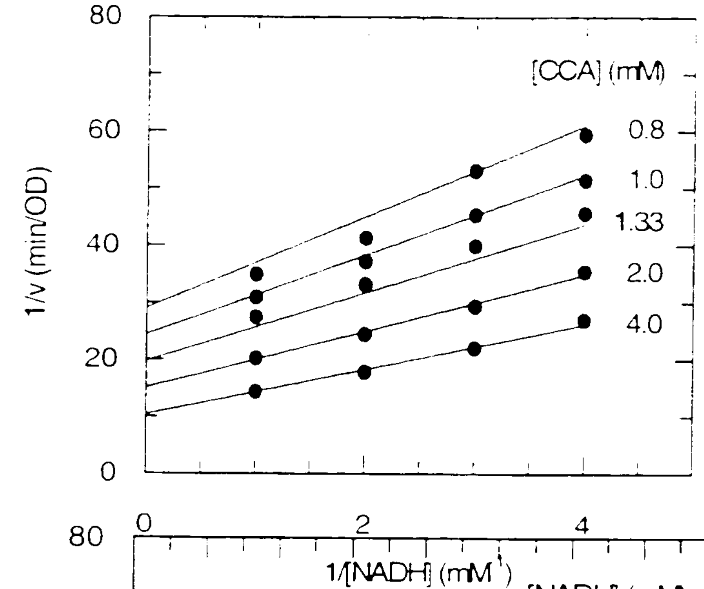
Figure 6. *S. aureus* Fab I Uses Both NADPH and NADH as Substrates

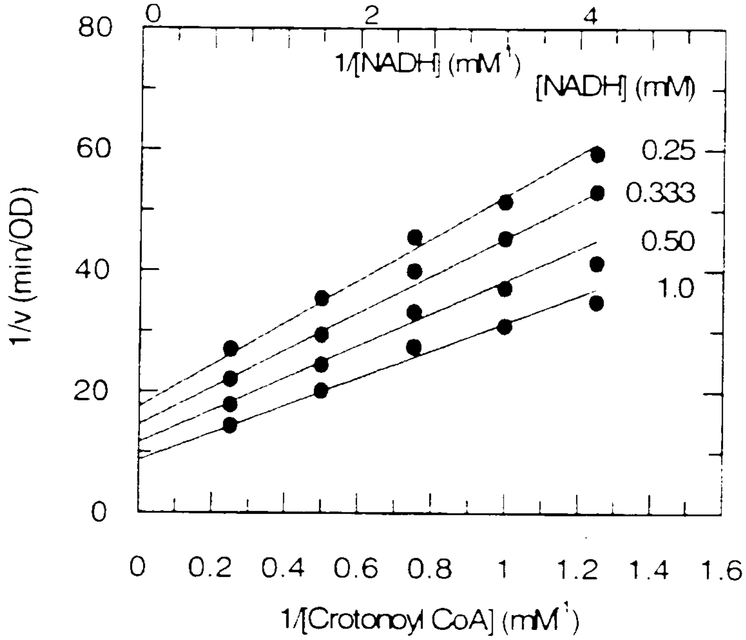




	NADH	NADPH
Vm(app) (µmol/min/mg)	3.75±0.23	1.21±0.06
Km(app) (mM)	0.41±0.06	0.013±0.002
Km(app) CCA (mM)	3.5±0.2	1.4±0.4

Figure 7. *S. aureus* Fab I Exhibits a Sequential Mechanism





$V_m = 0.18 \pm 0.02$
$K_a = 0.52 \pm 0.12$ mM
$K_b = 3.3 \pm 0.6$ mM
$K_{ia} = 0.22 \pm 0.06$ mM

Figure 8. pH Profile of *S. aureus* Fab I

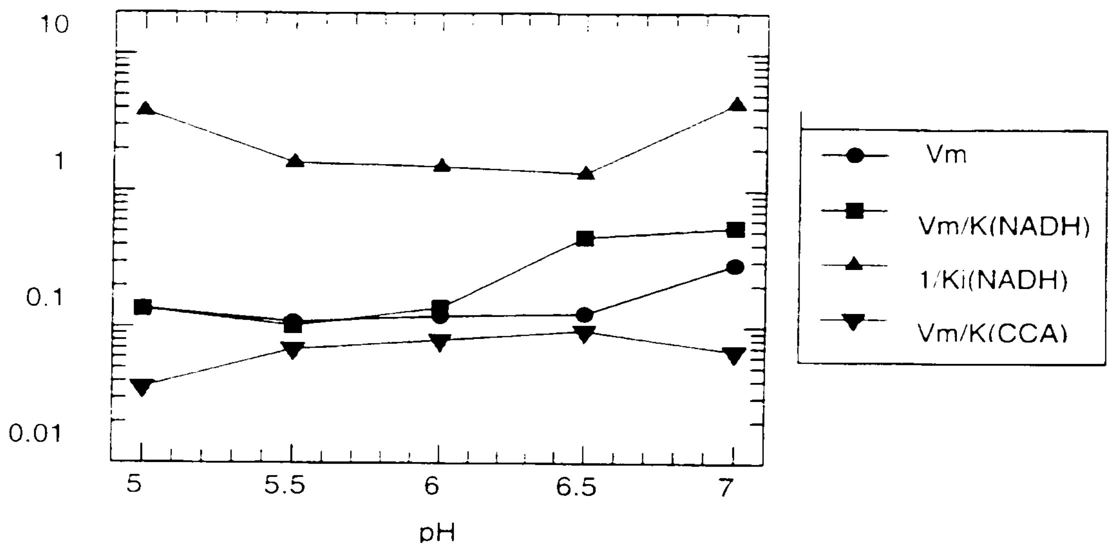


Figure 8 A Table 1. Inhibition by Saturated Fatty Acyl CoA's

CoA Derivative	*Mean IC_{50} (μM) (n=2)*
Acetyl	>>1000
n-Butyryl	>>1000
n-Hexanoyl	576
n-Octanoyl	248
n-Decanoyl	226
Lauroyl	48.4
Myristoyl	23.1
Palmitoyl	10.7

Figure 9. Inhibition by Palmitoyl CoA

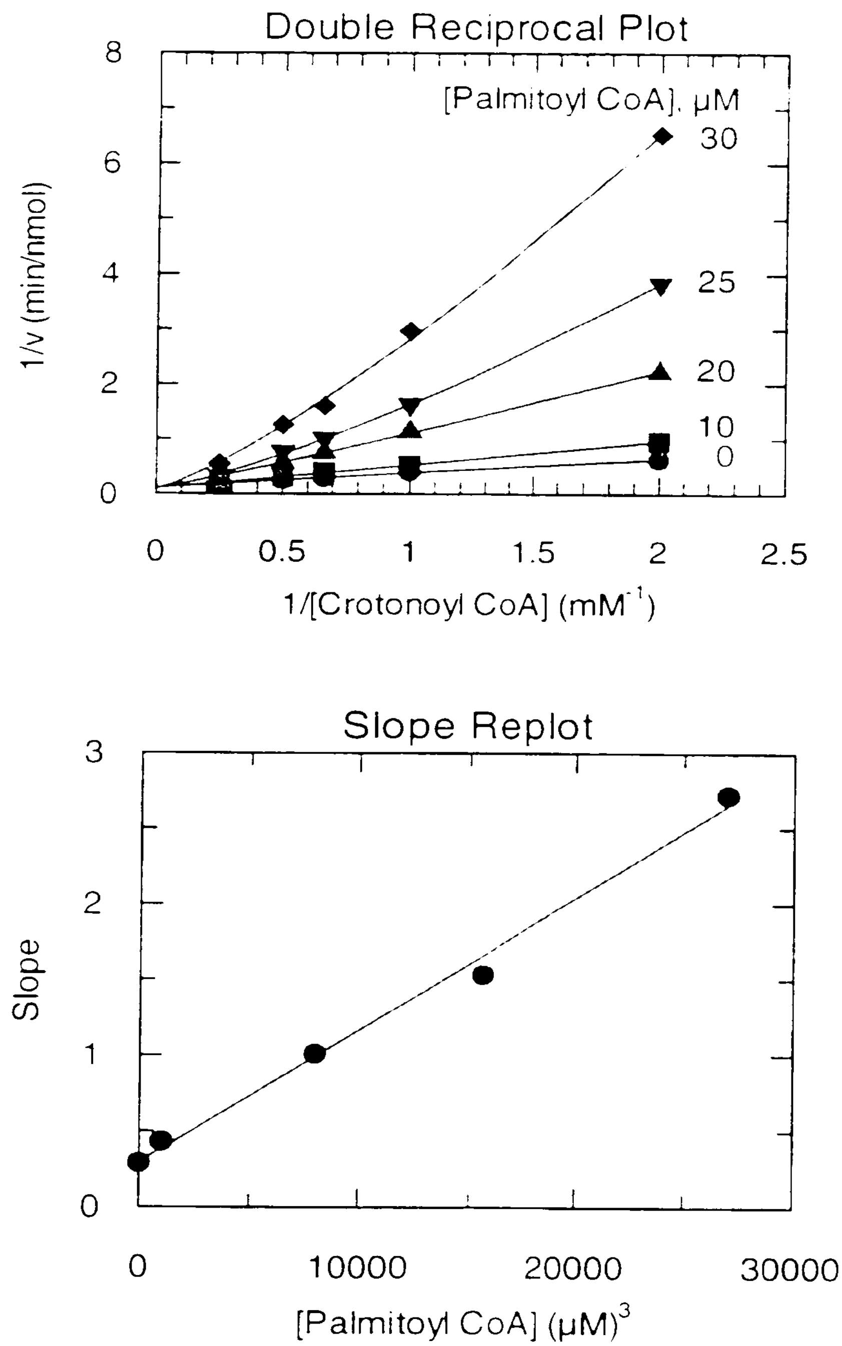
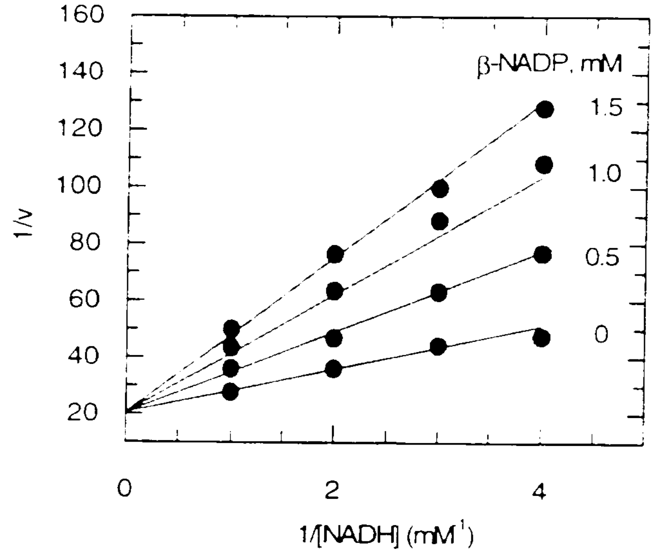
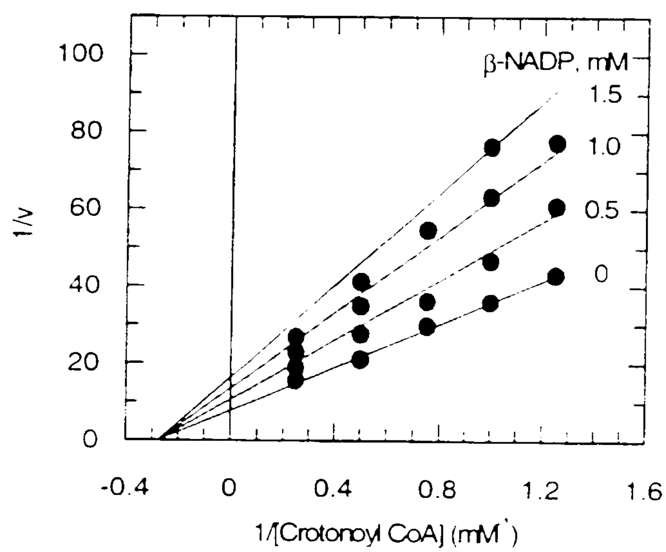


Figure 10. Inhibition by β-NADP+





Ki = 0.58±0.03 mM

Figure 11. Kinetic Model for Inhibition by β-NAPD+

$$
\begin{tikzcd}
E \arrow[r, shift left, "A"] \arrow[d, shift right, "I"'] & EA \arrow[l, shift left, "Kia"] \arrow[r, shift left, "B"] & EAB \arrow[l, shift left] \arrow[r] & EPQ \\
EI \arrow[u, shift right, "Ki"'] & & &
\end{tikzcd}
$$

$$v = \frac{V_m[A][B]}{(K_{ia}K_b + K_a[B])\left(1 + \frac{[I]}{K_i}\right) + K_b[A] + [A][B]}$$

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Figure 12. Inhibition by apo-ACP vs. NADH

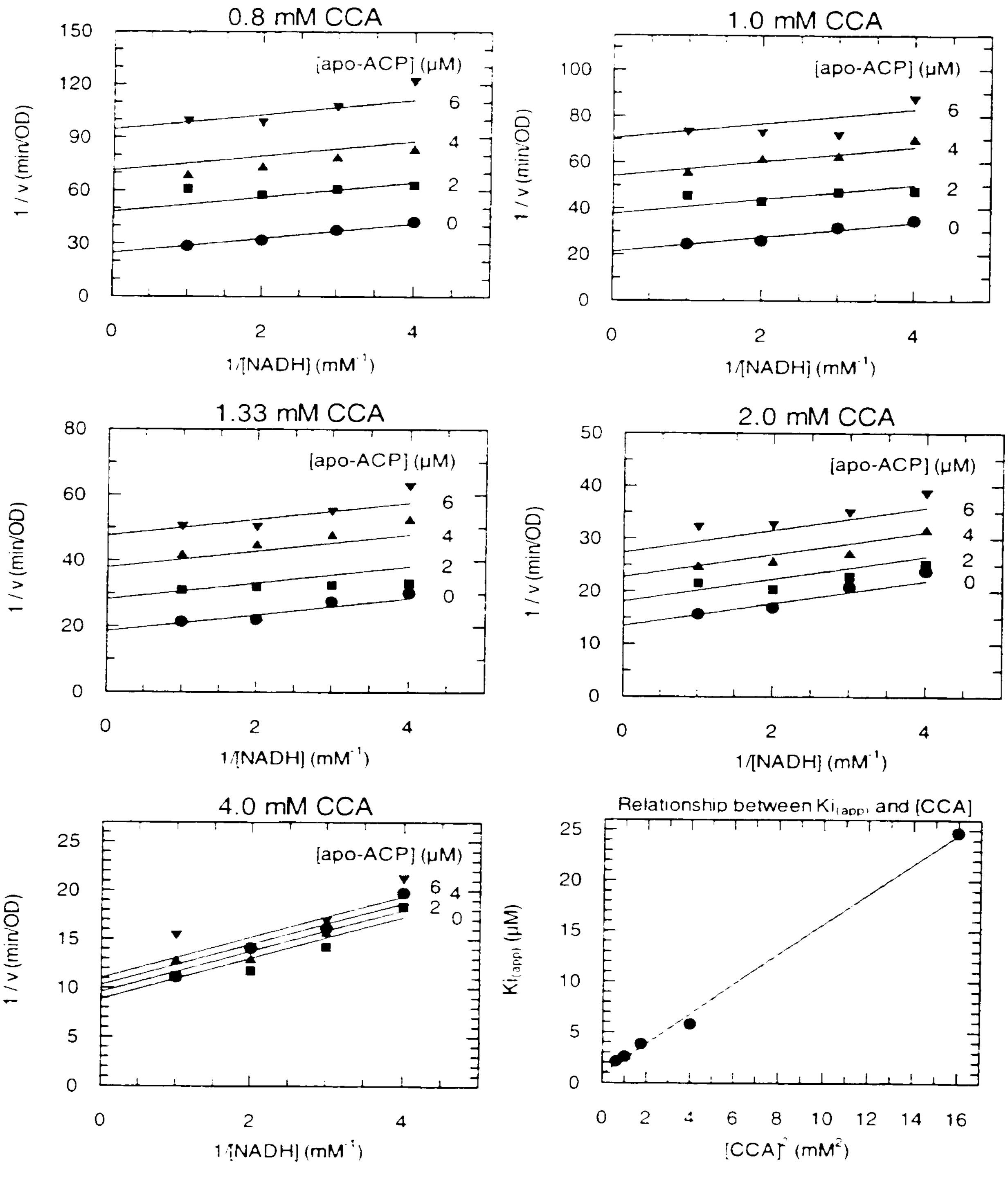

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Figure 13. Inhibition by apo-ACP vs. CCA

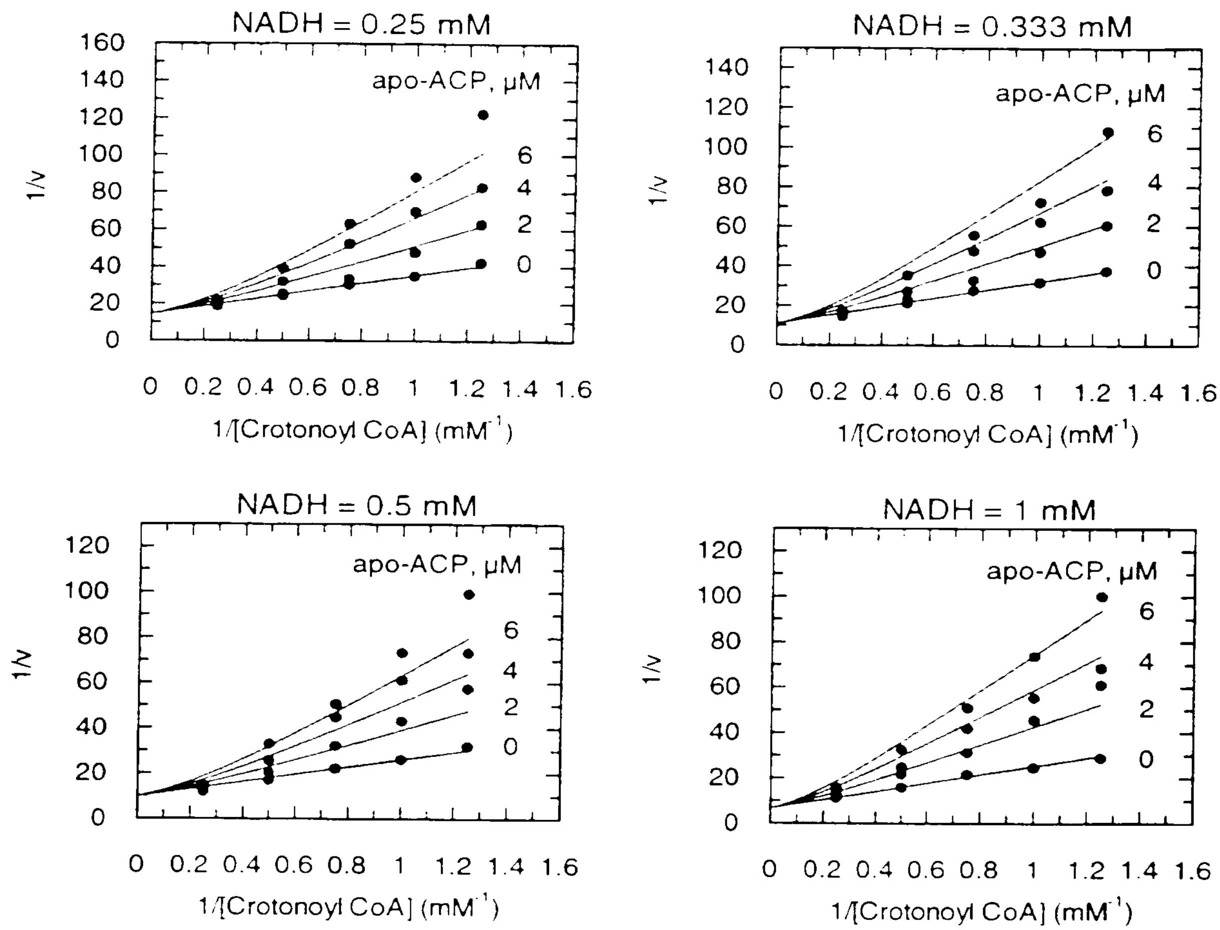

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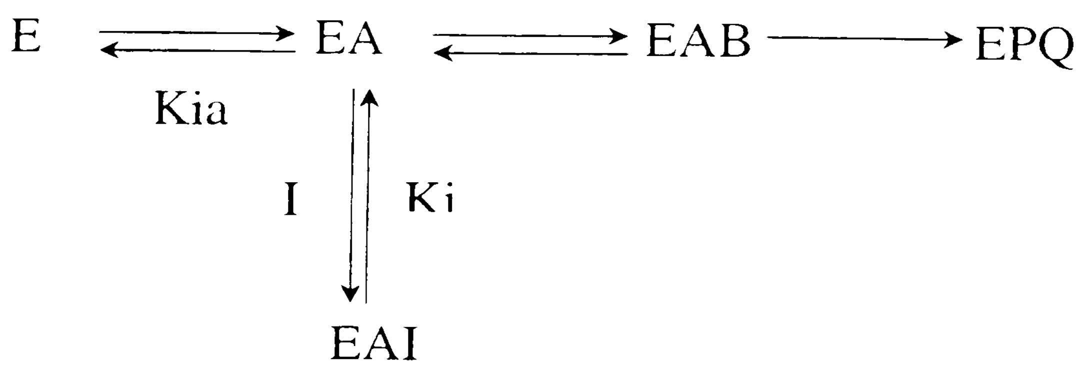
Figure 14. Minimal Kinetic Mechinism for Inhibition by apo-ACP



$$v = \frac{V_m[A][B]}{K_{ia}K_b + K_b[A]\left(1 + \frac{[I]}{K_i}\right) + K_a[B] + [A][B]}$$

Figure 15. Induced Cooperative Inhibition by apo-ACP

- Apo-ACP is uncompetitive versus NADH (Ki(app) and is proportional to the square of [CCA]).
- Apo-ACP is competitve versus crotonoyl CoA and induces negative cooperativivty with respect to Cca binding.

$$v=\frac{V_m\left[\frac{[S]}{K_S}+\frac{[S]^2}{K_S^2}+\frac{[S][I]}{\alpha K_S K_I}\right]}{\left[1+\frac{2[S]}{K_S}+\frac{[S]^2}{K_S^2}+\frac{2[I]}{K_I}+\frac{[I]^2}{K_I^2}+\frac{2[S][I]}{\alpha K_S K_I}\right]}$$

$$Ki = 3\ \mu M$$
$$\alpha = 15$$

FIGURE 16

[SEQ ID NO:2]

```
1    MLNLENKTYV IMGIANKRSI AFGVAKVLDQ LGAKLVFTYR KERSRKELEK
51   LLEQLNQPEA HLYQIDVQSD EEVINGFEQI GKDVGNIDGV YHSIAFANME
101  DLRGRFSETS REGFLLAQDI SSYSLTIVAH EAKKLMPEGG SIVATTYLGG
151  EFAVQNYNVM GVAKASLEAN VKYLALDLGP DNIRVNAISA GPIRTLSAKG
201  VGGFNTILKE IEERAPLKRN VDQVEVGKTA AYLLSDLSSG VTGENIHVDS
251  GFHAIK
```

FIGURE 17

[SEQ ID NO:1]

```
  1  ATGTTAAATC TTGAAAACAA AACATATGTC ATCATGGGAA TCGCTAATAA
 51  GCGTAGTATT GCTTTTGGTG TCGCTAAAGT TTTAGATCAA TTAGGTGCTA
101  AATTAGTATT TACTTACCGT AAAGAACGTA GCCGTAAAGA GCTTGAAAAA
151  TTATTAGAAC AATTAAATCA ACCAGAAGCG CACTTATATC AAATTGATGT
201  TCAAAGCGAT GAAGAGGTTA TTAATGGTTT TGAGCAAATT GGTAAAGATG
251  TTGGCAATAT TGATGGTGTA TATCATTCAA TCGCATTTGC TAATATGGAA
301  GACTTACGCG GACGCTTTTC TGAAACTTCA CGTGAAGGCT TCTTGTTAGC
351  TCAAGACATT AGTTCTTACT CATTAACAAT TGTGGCTCAT GAAGCTAAAA
401  AATTAATGCC AGAAGGTGGT AGCATTGTTG CAACAACATA TTTAGGTGGC
451  GAATTCGCAG TTCAAAATTA TAATGTGATG GGTGTTGCTA AAGCGAGCTT
501  AGAAGCAAAT GTTAAATATT TAGCATTAGA CTTAGGTCCT GATAATATTC
551  GCGTTAATGC AATTTCAGCT GGTCCAATCC GTACATTAAG TGCAAAAGGT
601  GTGGGTGGTT TCAATACAAT TCTTAAAGAA ATCGAAGAGC GTGCACCTTT
651  AAAACGTAAC GTTGATCAAG TAGAAGTAGG TAAAACAGCG GCTTACTTRT
701  TAAGTGACTT ATCAAGTGGC GTTACAGGTG AAAATATTCA TGTAGATAGC
751  GGATTCCACG CAATTAAATA A
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